NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 440

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF OZONE
(CAS NO. 10028-15-6)

AND

OZONE/NNK
(CAS NO. 10028-15-6/64091-91-4)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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(CAS NO. 10028-15-6)

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P.O. Box 12233
Research Triangle Park, NC 27709

October 1994

NTP TR 440

NIH Publication No. 95-3371

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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CONTENTS

ABSTRACT

O_3

OZONE

CAS No. 10028-15-6

Chemical Formula: O_3 Molecular Weight: 48

Synonym: Triatomic oxygen

There is widespread concern over the health effects of oxidant air pollutants. The state of California and the Health Effects Institute (HEI) (a nonprofit research institute funded jointly by the U.S. Environmental Protection Agency [USEPA] and combustion engine manufacturers) nominated ozone for evaluation in long-term animal studies. The NTP study designs were a result of a series of meetings at the NIEHS with scientists from NIEHS, USEPA, and HEI, as well as experts from academic institutions working in the area of air pollutants. Male and female F344/N rats and B6C3F$_1$ mice were exposed to ozone by inhalation for 4 weeks, 2 years, or for 124 weeks (rats) or 130 weeks (mice). The oxygen used to generate the ozone was greater than 99.9% pure. Additional groups of male F344/N rats were administered injections of 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (≥ 99% pure) 3 times per week for 20 weeks and exposed to ozone by inhalation for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*.

4-Week Ozone Study in Rats

Groups of five male and five female F344/N rats were exposed to 0, 0.5, or 1.0 ppm ozone by inhalation 6 hours per day, 5 days per week, for a total of 20 days. All rats survived to the end of the study. The final mean body weights and mean body weight gains of 0.5 ppm males and females and of 1.0 ppm females were similar to those of the controls. The final mean body weight of 1.0 ppm males was 7% lower than that of the controls. Clinical findings included hypoactivity in 1.0 ppm males and females and ruffled fur in exposed groups of males.

Male and female rats exposed to 0.5 or 1.0 ppm developed multifocal lesions of the lung, which consisted of infiltration of granulocytes and macrophages with extension of the bronchial epithelium into the alveolar ducts. Female rats exposed to ozone developed minimal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis.

Absolute and relative lung weights of all exposed groups of males and females were greater than those of the controls, and absolute and relative thymus weights of all exposed groups were generally lower than those of the controls.

4-Week Ozone Study in Mice

Groups of five male and five female B6C3F$_1$ mice were exposed to 0, 0.5, or 1.0 ppm ozone by inhalation 6 hours per day, 5 days per week, for a total of 20 days. All mice survived to the end of the study. The final mean body weights and body weight gains of all exposed groups of mice were less than those of the controls. Hypoactivity was observed in 1.0 ppm mice.

Male and female mice exposed to 0.5 or 1.0 ppm ozone developed patchy, multifocal lesions of the lung, which consisted of infiltration of granulocytes

and macrophages with extension of the bronchial epithelium into the alveolar ducts.

The relative lung weight of 1.0 ppm males was significantly greater than that of the controls. There were no other statistically significant differences in absolute or relative organ weights in males or females.

2-Year Ozone Study in Rats

The 2-year study was designed to include the present USEPA standard (0.12 ppm), the maximum concentration believed compatible with long-term survival (1.0 ppm), and an intermediate concentration (0.5 ppm). Groups of 50 male and 50 female F344/N rats were exposed to 0, 0.12, 0.5, or 1.0 ppm ozone by inhalation for 6 hours per day, 5 days per week, for 105 weeks.

Survival, Body Weights, and Clinical Findings

Survival of exposed groups of rats was similar to that of the controls at the end of the study. The mean body weights of 0.12 and 0.5 ppm males and females were similar to those of the controls throughout the study. The mean body weights of 1.0 ppm males and females were slightly lower than those of the controls throughout the study. Hypoactivity was observed in male and female rats exposed to ozone.

Pathology Findings

Increased incidences of ozone-induced metaplasia occurred in the nose and lung of rats exposed to 0.5 or 1.0 ppm ozone. The lesions in the nose were characterized by an increase in the number of goblet cells in the respiratory epithelium with mild squamous metaplasia of the cuboidal epithelium on the lateral wall. The increase in the number of goblet cells was found primarily in level I and II epithelium occurring along the lateral wall and on the maxilloturbinates and nasoturbinates. The metaplasia in the lung was a patchy multifocal lesion consisting of extension of the bronchial epithelium into the alveoli of the centriacinar region. This may represent more an extension of the bronchial epithelium into the pulmonary parenchyma than an actual transition of one epithelial cell type into another. There were increased incidences of squamous metaplasia at the base of the epiglottis characterized by one or more layers of flattened epithelial cells where low cuboidal cells are normally found.

There were no increases in the incidences of alveolar/bronchiolar adenoma or carcinoma in either males or females exposed to ozone.

Lifetime Ozone Study in Rats

For this study, rats were exposed to 0.5 and 1.0 ppm ozone for an additional 6 months to determine the effect of extended exposure on neoplasm incidence. Groups of 50 male and 50 female F344/N rats were exposed to 0, 0.5, or 1.0 ppm ozone by inhalation for 6 hours per day, 5 days per week, for 125 weeks.

Survival, Body Weights, and Clinical Findings

Survival rates of exposed rats were similar to those of the controls. The mean body weights of 0.5 ppm males and females were similar to those of the controls throughout the study. The mean body weights of 1.0 ppm males and females were slightly lower than those of the controls for the first two years of the study. Hypoactivity was observed in exposed groups of males and females.

Pathology Findings

Increased incidences of metaplasia occurred in the nose, larynx, and lung of rats exposed to 0.5 or 1.0 ppm ozone. The lung lesions were multifocal, centriacinar and were characterized by the presence of cuboidal epithelium (ciliated and nonciliated) along the alveolar ducts where type I epithelium is normally present. Inflammation (histiocytic infiltration) and interstitial fibrosis were observed in the lung of exposed males and females, and hyperplasia was observed in the nose of exposed male and female groups. There were no ozone-related increased incidences of neoplasms.

2-Year Ozone/NNK Study in Male Rats

An intermediate concentration of 0.5 ppm ozone was combined with exposure to two levels of a known carcinogen (0.1 and 1.0 mg NNK/kg body weight) in order to determine if ozone promotes the carcinogenic process or acts as a cocarcinogen. Groups of 48 male F344/N rats were exposed to 0 or 0.5 ppm ozone by inhalation, 6 hours per day, 5 days per week for 105 weeks. During the first 20 weeks of the study, these rats were subcutaneously injected with 0, 0.1, or 1.0 mg NNK per kg body weight in trioctanoin three times weekly.

Survival and Body Weights

Two-year survival rates of male rats were similar in all groups. Final mean body weights of all males exposed to NNK alone or NNK and ozone were similar to that of the controls, with the exception of rats exposed to 1.0 mg NNK/kg body weight and 0.5 ppm ozone. Hypoactivity was observed in males exposed to NNK and ozone, in those exposed to NNK without ozone, and in those exposed to ozone only.

Pathology Findings

Alveolar epithelial metaplasia and interstitial fibrosis occurred in all groups of rats exposed to ozone or to NNK and ozone, but not in those exposed to NNK without ozone. Increased incidences of hyperplasia occurred in groups of rats exposed to NNK or to ozone and NNK. Incidences of hyperplasia were similar among groups of rats exposed to NNK only. An increased incidence of alveolar/bronchiolar adenoma or carcinoma (combined) occurred in rats administered 1.0 mg/kg NNK, with or without ozone. The administration of ozone did not affect the occurrence of pulmonary neoplasms or nonneoplastic lesions in rats administered NNK.

2-YEAR OZONE STUDY IN MICE

The 2-year study was designed to include the present USEPA standard (0.12 ppm), the maximum concentration believed compatible with long-term survival (1.0 ppm), and an intermediate concentration (0.5 ppm). Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 0, 0.12, 0.5, or 1.0 ppm ozone by inhalation for 6 hours per day, 5 days per week, for 105 weeks.

Survival, Body Weights, and Clinical Findings

Survival rates of exposed mice were generally similar to those of the controls; the 2-year survival rate of 1.0 ppm females was greater than that of the controls. The mean body weights of 0.12 and 0.5 ppm males were similar to that of the controls throughout the study; the mean body weights of 1.0 ppm males and of all exposed groups of females were generally lower than those of the controls throughout the study. Hypoactivity was observed in male and female mice exposed to ozone.

Pathology Findings

Increased incidences of metaplasia occurred in the nose and lung of mice exposed to 0.5 or 1.0 ppm ozone. The metaplasia in the nose consisted of increased thickening and extension of the squamous epithelium in the anterior portion of the nasal passage. The metaplasia in the lung consisted of extension of the bronchial epithelium into the alveoli of the centriacinar region. There were increased incidences of hyperplasia in the nose characterized by thickening of the noncuboidal (transitional) epithelium. There were increased incidences of hyperplasia in the epiglottis of female mice, a change that was characterized by a minimal increase in the thickness of the epithelium.

Incidences of alveolar/bronchiolar adenoma or carcinoma (combined) were marginally increased in 0.5 and 1.0 ppm males (0 ppm, 14/50; 0.12 ppm, 13/50; 0.5 ppm, 18/50; 1.0 ppm, 19/50) and were increased in 1.0 ppm females (6/50, 7/50, 9/49, 16/50).

LIFETIME OZONE STUDY IN MICE

For this study, mice were exposed to 0.5 and 1.0 ppm ozone for 30 months to determine the effect of extended exposure on neoplasm incidence. Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 0, 0.5, or 1.0 ppm ozone by inhalation for 6 hours per day, 5 days per week, for 130 weeks.

Survival and Body Weights

Survival rates of exposed mice were similar to those of the controls. The mean body weights of 0.5 ppm males and females were similar to those of the controls throughout the study. The mean body weights of 1.0 ppm males and females were generally lower than those of the controls throughout the study. Hypoactivity was observed in male and female mice exposed to ozone.

Pathology Findings

The incidences of alveolar/bronchiolar adenoma and carcinoma (combined) were marginally increased in exposed males (0 ppm, 16/49; 0.5 ppm, 22/49; 1.0 ppm, 21/50) and in exposed females (6/50, 8/49, 12/50).

Increased incidences of metaplasia occurred in the nose, larynx, and lung of exposed groups of males and females, and the incidences of hyperplasia were increased in the larynx and nose of exposed mice. The morphology of the lesions was similar to that seen in the 2-year study. There were no ozone-related increases in alveolar epithelial hyperplasia.

GENETIC TOXICOLOGY

Ozone was mutagenic in *Salmonella typhimurium* strain TA102, with and without S9 metabolic activation.

CONCLUSIONS

Under the conditions of these 2-year and lifetime inhalation studies, there was *no evidence of carcinogenic activity** of ozone in male or female F344/N rats exposed to 0.12, 0.5, or 1.0 ppm. There was *equivocal evidence of carcinogenic activity* of ozone in male $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. There was *some evidence of carcinogenic activity* of ozone in female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma.

There was no evidence that exposure to 0.5 ppm ozone enhanced the incidence of NNK-induced pulmonary neoplasms in male rats.

Exposure of male and female rats to ozone for 2 years or 125 weeks was associated with goblet cell hyperplasia and squamous metaplasia in the nose, squamous metaplasia in the larynx, and metaplasia (extension of bronchial epithelium into the centriacinar alveolar ducts) and interstitial fibrosis in the lung. Exposure of male and female mice to ozone for 2 years or 130 weeks was associated with hyperplasia and squamous metaplasia in the nose and inflammation (histiocytic infiltration) and metaplasia (extension of bronchial epithelium into the centriacinar alveolar ducts) of the lung.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year and Lifetime Carcinogenesis and Genetic Toxicology Studies of Ozone

	Male F344/N Rats 2-Year Study	Male F344/N Rats Lifetime Study	Female F344/N Rats 2-Year Study	Female F344/N Rats Lifetime Study
Doses	0, 0.12, 0.5, or 1.0 ppm by inhalation	0, 0.5, or 1.0 ppm by inhalation	0, 0.12, 0.5, or 1.0 ppm by inhalation	0, 0.5, or 1.0 ppm by inhalation
Body weights	1.0 ppm group slightly lower than controls	1.0 ppm group lower than controls	1.0 ppm group slightly lower than controls	1.0 ppm group slightly lower than controls
Survival rates	8/49, 5/50, 7/50, 7/50	0/50, 0/50, 1/50	28/50, 24/50, 30/50, 27/50	6/50, 6/50, 7/50
Nonneoplastic effects	Nose: goblet cell hyperplasia (1/50, 4/50, 41/50, 48/50); lateral wall hyperplasia (0/50, 8/50, 50/50, 49/50) squamous metaplasia (2/50, 6/50, 36/50, 46/50) Larynx: squamous metaplasia (0/50, 2/50, 16/50, 43/50) Lung: metaplasia (0/50, 9/50, 46/50, 47/50); interstitial fibrosis (0/50, 2/50, 40/50, 44/50)	Nose: goblet cell hyperplasia (1/50, 46/49, 48/49); lateral wall hyperplasia (10/50, 48/49, 47/49); squamous metaplasia (10/50, 23/49, 40/49) Larynx: squamous metaplasia (0/50, 20/48, 43/47) Lung: metaplasia (0/50, 45/50, 50/50); histiocytic infiltration (0/50, 38/50, 49/50); interstitial fibrosis (0/50, 44/50, 50/50)	Nose: goblet cell hyperplasia (1/50, 2/50, 45/50, 50/50); lateral wall hyperplasia (2/50, 8/50, 48/50, 50/50) squamous metaplasia (2/50, 11/50, 21/50, 45/50) Larynx: squamous metaplasia (4/50, 5/50, 9/50, 43/50) Lung: metaplasia (0/50, 6/50, 48/50, 48/50); interstitial fibrosis (0/50, 0/50, 42/50, 47/50)	Nose: goblet cell hyperplasia (0/50, 47/49, 50/50); lateral wall hyperplasia (4/50, 49/49, 50/50); squamous metaplasia (5/50, 25/49, 35/50) Larynx: squamous metaplasia (2/49, 16/47, 48/50) Lung: metaplasia (0/50, 44/50, 50/50); histiocytic infiltration (0/50, 38/50, 49/50); interstitial fibrosis (0/50, 41/50, 50/50)
Neoplastic effects	None	None	None	None
Uncertain effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence		No evidence	

Summary of the 2-Year and Lifetime Carcinogenesis and Genetic Toxicology Studies of Ozone (continued)

	Male $B6C3F_1$ Mice 2-Year Study	Male $B6C3F_1$ Mice Lifetime Study	Female $B6C3F_1$ Mice 2-Year Study	Female $B6C3F_1$ Mice Lifetime Study
Doses	0, 0.12, 0.5, or 1.0 ppm by inhalation	0, 0.5, or 1.0 ppm by inhalation	0, 0.12, 0.5, or 1.0 ppm by inhalation	0, 0.5, or 1.0 ppm by inhalation
Body weights	1.0 ppm group slightly lower than controls	1.0 ppm group lower than controls	All exposed groups lower than controls	1.0 ppm group lower than controls
Survival rates	30/50, 34/50, 25/50, 27/50	14/50, 11/50, 12/50	29/50, 37/50, 33/48, 40/50	9/50, 12/50, 10/50
Nonneoplastic effects	Nose: hyperplasia (0/50, 0/50, 42/50, 50/50); squamous metaplasia (0/50, 3/50, 3/50, 36/50) Larynx: hyperplasia (1/50, 0/50, 0/50, 6/50) Lung: histiocytic infiltration (0/50, 0/50, 18/50, 31/50); metaplasia (0/50, 0/50, 48/50, 50/50)	Nose: hyperplasia (2/49, 33/48, 45/49); squamous metaplasia (1/49, 2/48, 20/49) Larynx: hyperplasia (4/49, 7/49, 15/50); squamous cell metaplasia (2/49, 1/49, 10/50) Lung: histiocytic infiltration (3/49, 40/49, 41/50); metaplasia (0/49, 48/49, 47/50)	Nose: hyperplasia (0/50, 0/50, 42/48, 50/50); squamous metaplasia (1/50, 1/50, 11/48, 36/50) Larynx: hyperplasia (0/50, 0/50, 0/49, 7/50) Lung: histiocytic infiltration (0/50, 0/50, 11/49, 42/50); metaplasia (0/50, 0/50, 43/49, 49/50)	Nose: hyperplasia (1/50, 42/49, 47/50); squamous metaplasia (2/50, 3/49, 28/50) Larynx: hyperplasia (13/50, 11/49, 24/50); squamous cell metaplasia (2/50, 2/49, 19/50) Lung: histiocytic infiltration (5/50, 39/49, 45/50); metaplasia (0/50, 43/49, 50/50)
Neoplastic effects	None	None	Lung: alveolar/ bronchiolar adenoma or carcinoma (combined) (6/50, 7/50, 9/49, 16/50)	Lung: alveolar/ bronchiolar adenoma or carcinoma (combined) (6/50, 8/49, 12/50)
Uncertain effects	Lung: alveolar/ bronchiolar adenoma or carcinoma (combined) (14/50, 13/50, 18/50, 19/50)	Lung: alveolar/ bronchiolar adenoma or carcinoma (combined) (16/49, 22/49, 21/50)	None	None
Level of evidence of carcinogenic activity	Equivocal evidence		Some evidence	
Genetic toxicology				
Salmonella typhimurium gene mutation:	Positive in strain TA102 with and without S9			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on Ozone and Ozone/NNK on November 16, 1993, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 16, 1993, the draft Technical Report on the toxicology and carcinogenesis studies of ozone and ozone/NNK received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. G.A. Boorman, NIEHS, introduced the toxicology and carcinogenesis studies of ozone and ozone/NNK by discussing the four basic studies: (1) 4-week studies in rats and mice; (2) the standard 2-year studies in rats and mice; (3) 30-month studies in rats and mice; and (4) a 2-year cocarcinogenesis or promotion study in male rats with NNK, a known carcinogen and tobacco-specific nitrosamine. He reported on survival and body weight effects and commented on the lack of neoplastic effects in male and female rats in the 2-year and 30-month studies and on compound-related neoplastic lesions in male and female mice in the 2-year and 30-month studies. Dr. Boorman discussed factors supporting or arguing against a compound-related carcinogenic effect in male and female mice. The proposed conclusions for the studies were: *no evidence of carcinogenic activity* of ozone in male and female F/344N rats; *equivocal evidence of carcinogenic activity* of ozone in male $B6C3F_1$ mice; and *some evidence of carcinogenic activity* of ozone in female $B6C3F_1$ mice.

Dr. van Zwieten, a principal reviewer, agreed with the proposed conclusions. He suggested that the Abstract should summarize pathology findings from the 4-week studies. He added that since the report documents a comprehensive series of studies with ozone, consideration should be given to including photomicrographs of ozone-induced lesions in the respiratory tract of rodents. Dr. Boorman agreed.

Dr. Bailey, the second principal reviewer, agreed with the proposed conclusions. He said the report indicated that "hypoactivity was observed in male and female rats exposed to ozone" and asked when the hypoactivity was seen. Dr. Boorman indicated that this occurred only during exposure and immediately afterwards.

Dr. Taylor, the third principal reviewer, stated that prior to the meeting he thought *equivocal evidence of carcinogenic activity* was more appropriate for female mice based on the relatively flat dose-response curve in the lifetime ozone studies. However, after looking at the combined data from the 2-year and lifetime studies, he supported the proposed conclusions in the report for female mice as well as the other proposed conclusions. Dr. J.K. Haseman, NIEHS, said there were two primary factors supporting *some evidence of carcinogenic activity* in female mice. One was that in the 2-year study there were 16 animals with alveolar/bronchiolar adenoma or carcinoma in the female 1.0 ppm group; this incidence was more than double the maximum seen historically in inhalation study controls. Second, in the analyses of the 2-year and lifetime studies (combined), the trend and the 1 ppm effects were an order of magnitude more significant in female mice than in male mice.

Dr. Ward questioned combining the conclusions in mice particularly since the incidence of alveolar/bronchiolar adenoma or carcinoma was higher in the 2-year study than in the lifetime study. Dr. Haseman responded that the combined analyses have the advantage of using all of the data, and because survival adjusted methods are used, animals are being compared to animals of equivalent age. Dr. Y. Vostal, Environmental Health Consultants, commented that a statement in the Introduction indicating that the primary source of ozone in urban areas was automotive emissions was incorrect.

Dr. van Zwieten moved that the Technical Report on ozone and ozone/NNK be accepted with the revisions discussed and with the conclusions that there was *no evidence of carcinogenic activity* for male and female rats, *equivocal evidence of carcinogenic activity* for male mice, and *some evidence of carcinogenic activity* for female mice. Dr. Taylor seconded the motion, which was accepted by four yes votes with one abstention (Dr. Ryan).